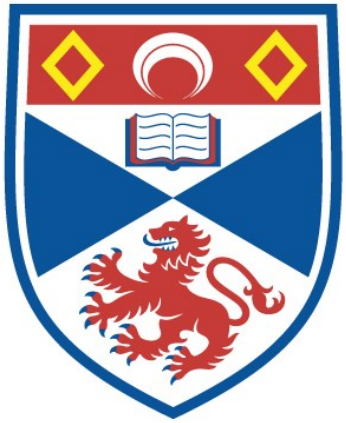
THE MOLECULAR CONSTITUTION OF INULIN

George McOwan

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1923

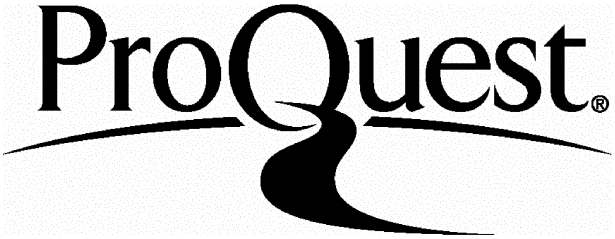
THE MOLECULAR CONSTITUTION OF INULIN.

being a Thesis presented by

GEORGE McOWAN, M.A.B.Sc.

to the University of St.Andrews

in application for the degree of Ph.D.



DECLARATION.

I hereby declare that the following Thesis is based on the results of experiments carried out by me, that the thesis is my own composition, and that it has not previously been presented for a Higher Degree.

The research was carried out in the Chemical Research Laboratory of St. Andrews, under the direction of Principal Irvine, C.B.E., D.Sc., LL.D., F.R.S.

The following abbreviations are used in referring to Journals:-

J.C.S.....Journal of the Chemical Society. Trans.

J.A.C.S.....Journal of the AmericanChemical Society.

Ann.....Justus Liebig's Annalen der Chemie.

Ber.....Berichte der Deutschen Chemischen Gesellschaft.

C.R.....Comptes Rendus.

Rec.trav.Chem.....Receuil des travaux chemiques des Pays-Bas et de la Belgique.

Helv.chim.Acta.....Helvetica Chemica Acta.

CERTIFICATE.

I certify that Mr.G.McOwan M.A.B.Sc.has spent nine terms at research work under my direction and that he has fulfilled the conditions of Ordinance No.16 (St.Andrews) and is qualified to submit the accompanying Thesis in application for the Degree of Ph.D.

Principal and Vice Chancellor.

St.Andrews,
April 1923.

CAREER.

I matriculated in the University of St.Andrews in October 1912 and followed a course leading to graduation in Arts and Science until 1914,when I was gazetted to a Commision in the Army.

On demobilisation in January 1919,I resumed my studies and completed my degree in Arts and Science In 1920 I became attached,as a research chemist,tothe Fuel Research Board but resigned this appointment on being awarded a Carnegie Research Scholarship.In this capacity I undertook the research on inulin which is now being submitted as a Ph.D.Thesis

CONTENTS.

PART I.

INTRODUCTION.

The investigation on inulin,which forms the subject of the present thesis,is an extension of the earlier work of Irvine and Steele,(T.1920,117,1474), who placed the study of this polysaccharide on an exact basis.These authors proved,

(a) that inulin is composed entirely of anhydro-fructose residues in which each ketose unit has lost two hydroxyl groups,

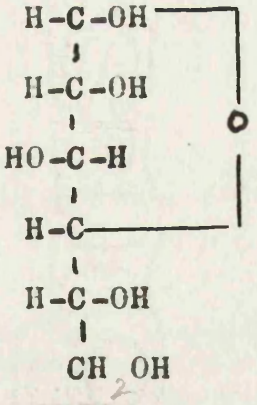
(b) that all the fructose groups in inulin belong to the γ- series.

In consequence of these important discoveries a special interest is attached to inulin,which is unique among natural compounds in being based entirely on a γ-sugar.

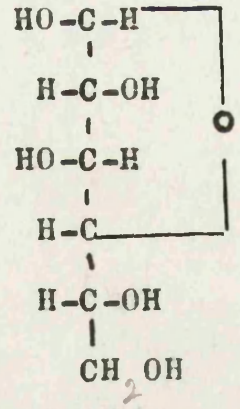
As the experimental development of the subject is of necessity concerned with the reactions and structure of γ-fructose,it is advisable to include in the introduction an account of the discovery and properties of γ-sugars generally.

From the fact that glucose does not exhibit many of the characteristic properties of aliphatic aldehydes,Tollens,(Ber.1883,16,921) proposed a cyclic

structure for the reducing sugars in preference to the previously accepted hydroxy-aldehyde constitution. This proposal which, for various reasons, came to be regarded as involving a 1-4 or butylene-oxide linkage, met with general acceptance and Simon, (C.R.1901,132,487,596), later made use of it to show that, on this basis, a satisfactory explanation could be given for the existence of the well known α and β isomerides of glucose. According to Simon the stereoisomerides are,



α Glucose.

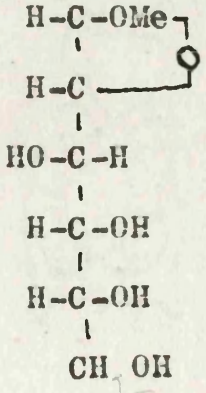


β Glucose.

This view, despite an attempt by Nef, (Ann.1914,204), to formulate β glucose as possessing a propylene-oxide linkage, an opinion which was disproved by Fischer (Ber.1914,47,1980), was supported by Fischer Irvine and Boesecken.

The question of the nature of the linking in sugars thus appeared to be settled until Fischer (Ber.1914,47,1980) discovered a reactive form of

methylglucoside differing from the α and β methylglucosides. The compound was termed γ-methylglucoside and the expression "γ sugar" has come into use to indicate aldoses and ketoses which do not conform to the normal type. Irvine,(J.C.S.1913,1896),had simultaneously made the same discovery and later,(Irvine,Fyfe and Hogg, J.C.S.1915,108,524),examined the tetramethyl γ-glucose obtained from the new isomeride and so indicated its structure.The new type of isomerism must be due to a displacement of the oxygen link from its normal 1-4 position to one of the three positions 1-2,1-3 or1-5. The weight of the evidence available at the time was in favour of the ethylene-oxide linking and the following formula was proposed by Irvine as a tentative representation of γ-methylglucoside,



The existence of such isomerism of the γ-type has been found not to be confined to glucose but has

been detected in other aldoses.Cunningham,(J.C.S.1918,113,596) working on the methylgalactosides,obtained a compound showing the properties of a γ-sugar and Hudson, (J.A.C.S.1916,38,1223),has succeeded in preparing all four crystalline pentacetates of galactose,two being related to the normal α and β galactoses and the remaining two to the new variety of galactose.Armstrong,from his researches on the discolouration of dilute potassium permanganate by sugars dissolved in dilute acids,has come to the same conclusion regarding several hexoses and pentoses.

Considering the above discoveries we should naturally expect that fructose,in common with other hexoses,would be capable of reacting in the γ- form.From results obtained in the preparation of methylated derivatives of fructose,using methylfructoside prepared according to Fischer's method,(Ber.1895,28,1160), Purdie,(J.C.S.1907,91,289),though the discovery of sugars of the 'γ' type had not then been made,predicted the existence of a third dextro-rotatory product in addition to the methylfructosides already known.Working in the light of later knowledge,Irvine and Robertson, (J.C.S.1916,109,1305),afforded a complete explanation of what had necessarily beenobscure in the earlier work

of Purdie and showed that, during the condensation of fructose with methyl alcohol containing 0.5% of hydrogen chloride, two simultaneous reactions take place and the products of the reaction contain a derivative of γ-fructose.

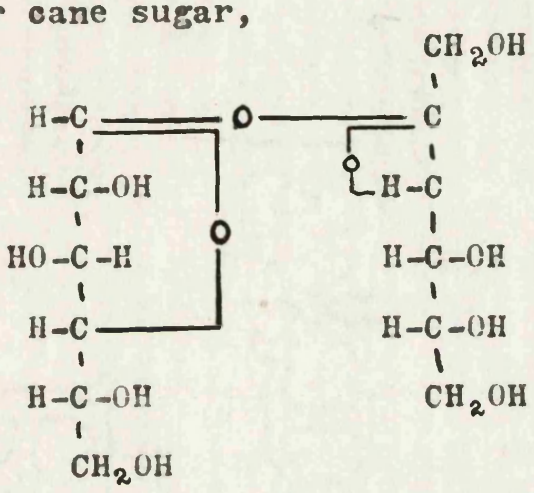
Most of the earlier researches conducted in the St. Andrews' laboratories, on subjects connected with sugar chemistry, were completed with a view to preparing methylated derivatives of simple sugars of known constitution, to be utilised as standard substances for reference when it would be possible to examine the constitutions of the naturally occurring glucosides and polysaccharides. The methods employed by Irvine in these laboratories are too well known to require either elaboration or explanation here, beyond stating that the free hydroxyl groups in a disaccharide or polysaccharide can be replaced by stable methoxyl groups and that on hydrolsis a methylated sugar is obtained from which, as the possibility of a displacement of the oxygen linking has been excluded, conclusions can be drawn as to the constitution of the parent substance.

After the necessary preliminary investigations had been carried out on γ-fructose, the question naturally arose whether such residues play a part in the formation of the natural complex .

carbohydrates.Hydrolysis of these by acids in the usual manner fails to reveal any trace of γ-residues,as these being unstable,would be converted into the more stable isomeride.Attention was,however,directed to the fact that naturally-occurring carbohydrates containing fructose were hydrolysed by acids under the same conditions as γ-sugar derivatives.

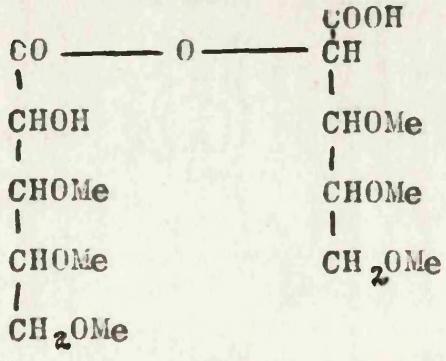
Amongst these,as is well known,is sucrose,which,from its importance and accessibility, was the first to come under investigation.Purdie and Irvine,(J.C.S.1905,87,1022),by the methylation of sucrose obtained octamethyl sucrose,which on hydrolysis failed to show the optical inversion characteristic of the parent sugar.Hudson,(J.A.C.S.1909,31,660),confirmed the presence of a dextro-rotatory fructose residue by the study of the hydrolysis of sucrose by invertase.Under these conditions he showed that the glucose constituent was liberated in the α-form and calculated the rotation of the fructose residue as + 17°.Later Haworth (J.C.S.1916,109,1314),succeeded in isolating tetramethyl γ-fructose from the products of hydrolysis of heptamethyl sucrose and adopting the suggested ethylene-oxide linking for γ-fructose put forward the following

formula for cane sugar,



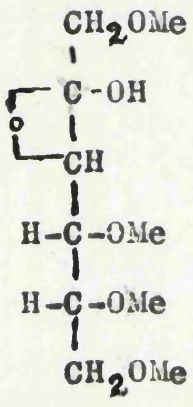
The position of the oxygen link had,however, not been definitely determined.Two researches,based on the oxidation of tetramethyl fructose and recognition of the acid formed,were undertaken with a view to settling this problem.The results of these investigations lead to different conclusions being drawn.For purposes of comparison these are tabulated below.

(a) Haworth(J.C.S.1920,117,199),after oxidising tetramethyl γ-fructose with nitric acid,identified his product as the semi-lactide of trimethoxy valeric acid,



and thus deduced the following constitution for

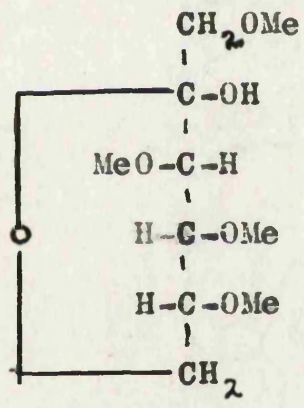
tetramethyl fructose,



(b) Mitchell,(Ph.D.Thesis,St.Andrews University), (later Haworth and Mitchell,J.C.S.1923,123,301),after oxidising tetramethyl γ-fructose with alkaline potassium permanganate,isolated a dimethoxy butyro-lactone and, after examining the tetramethyl hexitols produced on reduction of tetramethyl γ-fructose,proposed a 1-3 or 1-5 cyclic structure for γ-fructose.

Since this present research was undertaken, Haworth,(J.C.S.1923,123,294),has published the results of a further oxidation with nitric acid.The product isolated was trimethoxy valero-lactone which was subsequently converted into trimethoxy glutaric anhydride,by oxidising with alkaline potassium permanganate From the anhydride the dimethyl ester of trimethoxy glutaric acid was prepared.Onthis basis tetramethyl

γ-fructose possesses an amylene-oxide ring which, remaining undisturbed during the oxidation, passed to the analogous δ-lactone structure, which was then resistant to oxidation at a point where a potential primary alcohol group existed. The following formula was therefore proposed by Haworth for tetramethyl γ-fructose.



From reasons similar to those, which led to the investigation of sucrose, the problem of the elucidation of the constitution of inulin was entered upon.

Irvine and Steele, (J.C.S. 1920, 117, 1474), applying the methods customary in the St. Andrews laboratories, showed that inulin, on methylation with methyl sulphate and sodium hydroxide, gave as the main product dimethyl inulin and that, when the methylation was continued with silver oxide and methyl iodide, a quantitative yield of a trimethyl inulin was obtained. This, on hydrolysis, was converted into trimethyl γ-fructose. They succeeded in showing that this substance on further

methylation gave the same tetramethyl γ-fructose as was obtained from sucrose.Later Karrer and Lang (Helv.Chim.Acta, 1921,IV,249),encroaching upon this field of work and utilising the same methods,methylated inulin and obtained a trimethyl derivative differing from that which had been earlier described.Irvine and Steele (loc.cit.) obtained as the final product of methylation a flexible glass $[\alpha]_D = +55.6^\circ$ and comment on the remarkable change of rotation, $[\alpha]_D = -42.1^\circ$ to $[\alpha]_D = +55.6^\circ$, which is caused by the transition from dimethyl inulin to the trimethyl compound. Karrer and Lang,(loc.cit.),obtained as their product a colourless,amorphous solid $[\alpha]_D = -42.3^\circ$.Despite the fact that Irvine and Steele had previously shown inulin to consist of γ-fructose residues identical with that present in sucrose,each ketose molecule having lost two hydroxyl groups in the formation of the polysaccharide, Karrer advances the statement that the question is still undecided as to which anhydro-sugar plays a part in the formation of inulin.

In a later contribution to the subject Irvine,Steele and Shannon,(J.C.S.1922,121,1060),the earlier statement that,after methylating with methyl sulphate and sodium hydroxide,the characteristic product was dimethyl inulin,was upheld and it was shown that,on

completing the methylation,the physical properties of trimethyl inulin varied in marked degree according to the experimental procedure adopted in preparing the compound,the gradation in optical activity being accompanied by a corresponding difference in solubility. Three varieties of trimethyl inulin were described,

(a) soluble in ether, $[\alpha]_D = +59.2^\circ$

(b) soluble in ether, $[\alpha]_D = -46.62^\circ$

(c) insoluble in ether, $[\alpha]_D = -49.25^\circ$

Irvine accounts for these differences by depolymerisation,the more highly polymerised product possessing the higher rotation in the laevo sense and less solubility generally in organic solvents.Though the magnitude of the rotation of trimethyl inulin does not affect the validity of the views expressed by Irvine regarding the constitution of inulin,the problem has recently become of importance from researches on allied topics.From results obtained in the methylation of starch Irvine,(Britt.Ass.Reports,1922),showed that the simplest unit in starch could be regarded as a trisaccharide and proposed a formula for this compound.Later Pictet, (Helv.Chim.Acta.1922,V,640),by heating starch in glycerol solution,isolated an anhydro trisaccharide,differing from the triamylose of Pringsheim (Ber.1922,55,1433),

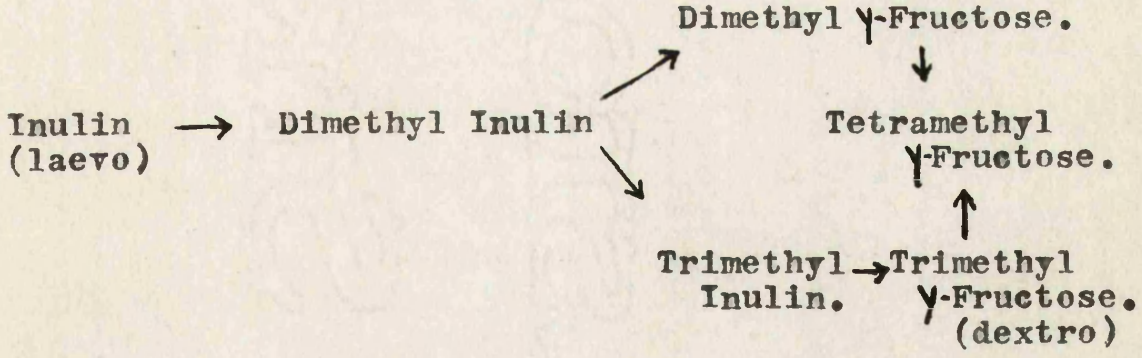
and from the hexamylose of Karrer(Helv.Chim.Acta.1922, I,435),and there may be a relation between this and the trisaccharide of Irvine.The possibility is thus not excluded that,by the methylation process yielding a laevo-rotatory trimethyl inulin,the depolymerisation process has not been complete,a possibility which Karrer, though offering criticisms of the results of Irvine and Steele,is forced to admit.

It is interesting,in view of these results, to make reference to later work.Pringsheim,(Ber.1922,55, B.1414-1425),has indicated,though the evidence is not conclusive,that inulin on treatment with phenyl hydrazine acetate gives,in addition to glucosazone traces of a second osazone soluble in hot water.In addition the same investigator has found that,when inulin acetate is treated with sodium ethoxide,an additive compound possessing the formula $(C_6H_{10}O_5)_3.NaOH$ is obtained.He therefore concludes that inulin in the solid state and in colloidal condition is a product of a polymerised anhydro-trifructose.

The degree of polymerisation he is able to deduce from the molecular weight of inulin triacetate, (Pringsheim and Aronowsky,Ber.1921,54,1281),which was found to be 2600,from which it follows that inulin consists

of nine fructose residues.This is in agreement with Karrer and Lang,whose values for the molecular weight of trimethyl inulin,showed it to consist of 8 to 10 fructose residues.

When the present research was commenced, the position which had been reached may be synopsised in the scheme shown below.



Obviously,if an exact constitutional formula can be ascribed to the ultimate product, trimethyl γ-fructose,the molecular structure of inulin can be deduced.All that was known of the trimethyl fructose was that it belonged to the γ-series and was convertible into tetramethyl γ-fructose but,as already explained,the exact constitution of the latter was uncertain.In addition it may be pointed out that no evidence was available as to whether the trimethyl γ-fructose was a single chemical individual or a mixture of isomerides derived from

γ - fructose. In other words, no symmetry had been established for the molecular unit of inulin.

PART II.

OUTLINE

of Methods and Results.

The trimethyl inulin used in these researches was prepared in quantity by methylating the polysaccharide twice with methyl sulphate and sodium hydroxide,when the product was found to have a methoxyl content in the neighbourhood of 39% and to be soluble in metthyl iodide.The methylation was thereafter completed by treatment with silver oxide and methyl iodide.The product,which was isolated by extracting with boiling alcohol,settled out on cooling as a white, amorphous powder and was thus obtained free from fructose The results of different preparations are set forth in the experimental part.They are here compared with,

(A) Results by Irvine and Steele.(J.C.S.1920,117,1474)

(B) Results by Karrer and Lang.(Helv.Chim.Acta, 1921,IV,249)

(C) Results by Irvine,Steele and Shannon. (J.C.S.1922,121,1060)

	A.	B.	C.	D.
After one methylation with methyl sulphate & sodium hydroxide.	OMe=32.7% &27.6%			
After two methylations with methyl sulphate and sodium hydroxide.		OMe=39%-39.7%	OMe=39%	OMe=37.6% -40.02%
Trimethyl inulin.	= 55.6 & 59.2	42.4 & 43	= 46.62 & 49.25	39.8 & 50.85
Solubility of trimethyl inulin.				
Soluble:-	Alcohol, chloroform & acetone.	Organic solvents generally.	Alcohol, part in ether.	Chloroform.
Sparingly soluble	Water, ether.	Water		Alcohol, ether & water.

There is thus a general agreement in the rotations of the trimethyl inulin in B,C and D,though there are,as has already been indicated by Irvine, (loc.cit.),discrepancies in these and in the solubilities of the products.The significance of these and also of the dextro form A are discussed in part IV of this paper.

Attempts to convert the form of trimethyl inulin obtained here to that described by Irvine and Steele were carried out.It was thought at first that the change in rotation of the earlier preparation from laevo to dextro had been occasioned by some factor in the methods of extraction and purification used.An exhaustive examination was therefore made,in which the effect of various solvents,of decolourising charcoal and of heating both below and above the melting point was examined.No marked change in rotation,however,was observed and in every case the substance was recovered unchanged.The time required for complete hydrolysis with 1% oxalic acid in 99% ethyl alcohol having been found to be 30 hours,it was found that,when the reaction was stopped before the hydrolysis was complete,a small amount of a viscous syrup was invariably obtained.This,after the removal of the methylated fructose products of hydrolysis, had a higher dextro rotation $[\alpha]_D = +39^\circ$ to $+47^\circ$ than

trimethyl γ-fructose, effected but little reduction of Fehling's solution and had the methoxyl content required for a trimethyl inulin. A significant fact is that this value was not increased by the action of silver oxide and methyl iodide; whereas, had the product consisted of methylated fructoses, this value would have been substantially increased. (Trimethyl inulin requires OMe=45.8%; Tetramethyl methylfructoside requires OMe=62%) There are thus good grounds for believing that the substance so obtained is a dextro-rotatory trimethyl inulin and that the first stage in effecting hydrolysis is the depolymerisation of the original substance.

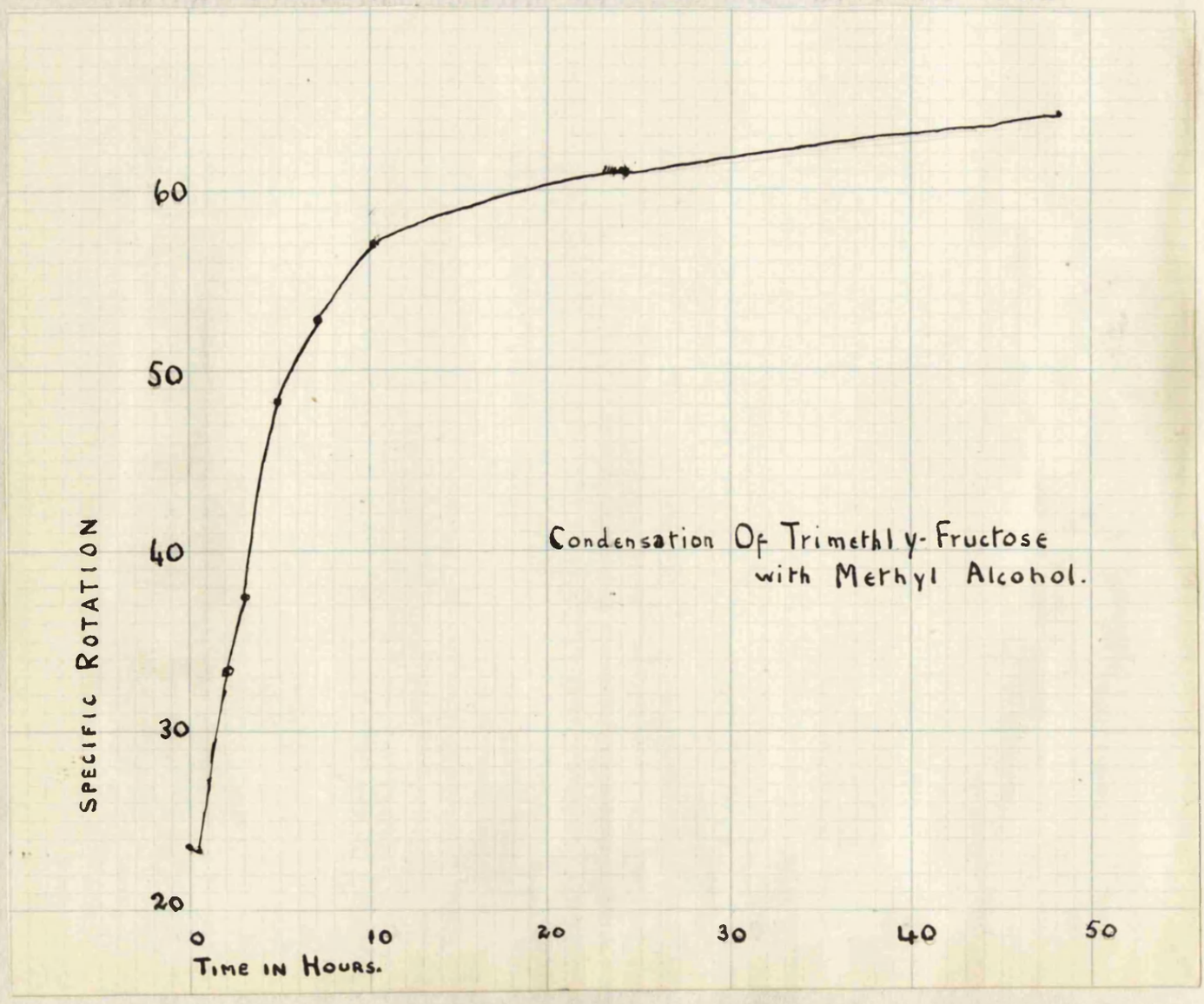
In addition to trimethyl inulin, the yield of which varied between 65% and 75% of that required by theory, there was isolated in two cases a quantity of syrup varying in amount, which was found to consist of a mixture of trimethyl inulin and fully methylated fructose. Mixed with this were other degradation products of fructose, probably arising from the hydrolysis of inulin during methylation and subsequent resinification of the fructose formed by the sodium hydroxide. This result has no bearing on the main topic of research. The product was isolated in varying amounts; in the first preparation practically no derivatives of fructose were obtained.

In the case of such an easily hydrolysed compound as inulin, it is to be expected that, unless exceptional precautions are taken to prevent the development of local acidity during the methylation process, some fructose and methylated derivatives of fructose will be formed. This had occurred in the earlier preparations of Irvine and Steele and of Irvine, Steele and Shannon. In this case no steps were taken to remove these degradationproducts until the final and desired product, trimethyl inulin, was isolated in the pure state and according to expectation some fructose, which had been completely methylated, was obtained as a by-product of the reactions.

It was shown that a trimethyl inulin with the above properties yielded, on hydrolysis with oxalic acid, the same trimethyl γ-fructose as had been obtained by Irvine and Steele and by Irvine, Steele and Shannon. A comparison of these products is given below:-

	Irvine & Steele	Irvine, Steele & Shannon	Irvine & McOwan
$[\alpha]_D$ in chloroform	26.61°	24.8°	28.5°
B.Pt.	146°/ 0.37 mm.		145°/0.4 mm.
n_D	1.4689		1.4686

The properties ascribed to the sugar were confirmed, including the fact that no crystalline


60
50
40
30
20
SPECIFIC ROTATION
0
10
20
30
40
50
TIME IN HOURS.
Condensation Of Trimethly-Fructose
with Methyl Alcohol.

osazone was formed by the action of phenyl hydrazine and acetic acid.

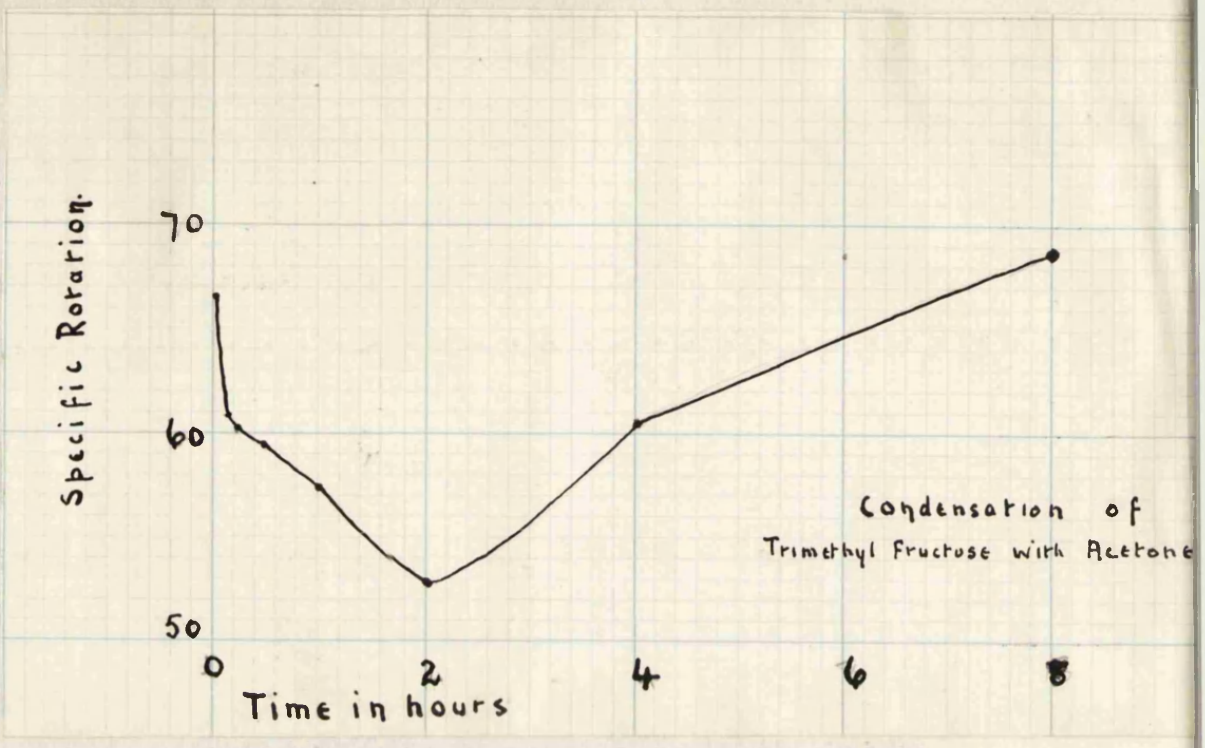
Much work was devoted to proving that only one trimethyl γ-fructose is obtained on the hydrolysis of trimethyl inulin,a result which establishes the symmetry of the inulin molecule.As the methylated ketose is a liquid,from which no crystalline derivatives have so far been obtained,the proof of its homogeneity had to be adduced from careful fractionation and a study of the rotations and rates of reaction of the various fractions collected.Since,however,trimethyl fructose is fairly viscous and considerable loss would have been involved on distillation,it was resolved to carry out this part of the work with trimethyl γ-methylfructoside,which distills more readily and admits of more careful fractionation.

This substance can be and was prepared by dissolving trimethyl γ-fructose in methyl alcohol containing 0.25% of hydrogen chloride but in general the hydrolysis of trimethyl inulin and condensation with methyl alcohol were effected simultaneously.For this purpose trimethyl inulin was heated in methyl alcohol containing 0.5% of hydrogen chloride at the

boiling point.As the material went slowly into solution in the initial stage,and was reprecipated on cooling, the course of the reaction could not be followed polarimetrically but a constant reading $[\alpha]_D = +66.25^\circ$ was obtained after $1\frac{1}{2}$ hours heating.This preparation of trimethyl γ-methylfructoside would consist of a mixture of α and β isomerides in unknown proportions.It was a dextro-rotatory syrup,(B.P.108°-110°/0.3 mm.and n_D=1.4574)-which did not reduce Fehling's solution until after hydrolysis.

Despite the careful fractionation of the trimethyl γ-methylfructoside so prepared,no difference was detected in the physical constants of the individual fractions.This in itself offers powerful evidence in favour of the view that,save for α and β isomerism,the compound was homogeneous.Confirmation was,however, provided by means of an obscure but extremely interesting property of γ-glucosides.

In an attempt to convert monomethyl methylfructoside into monomethyl methylfructoside-mono-acetone,Irvine and Hynd,(J.C.S.1909,95,1220),found that, when dissolved in acidified acetone,the fructoside lost the labile methyl group.This change was accompanied by condensation with the solvent acetone,so that


Specific Rotation
70
60
50
0
2
4
6
8
Time in hours
Condensation of
Trimethyl Fructose with Acetone

monomethyl fructose-diacetone resulted.

As it had been observed,(Irvine and Steele, loc.cit.),that trimethyl γ-fructose condensed with acetone,a similar reaction was undertaken in the present instance starting from the fructoside.A 2% solution of trimethyl methylfructoside was made in acetone containing 0.2% of hydrogen chloride.The polarimetric curve of the reaction,when the time in hours is plotted against the specific rotation,is shown opposite.As was expected, the substance on isolation was found to be trimethyl γ-fructosemonoacetone.(B.P.95°/0.42 mm., $[\alpha]_D = +69.77°$) It was further shown that the acetone derivative on hydrolysis with aqueous hydrochloric acid,was converted into trimethyl γ-fructose, $[\alpha]_D = +26.54°$

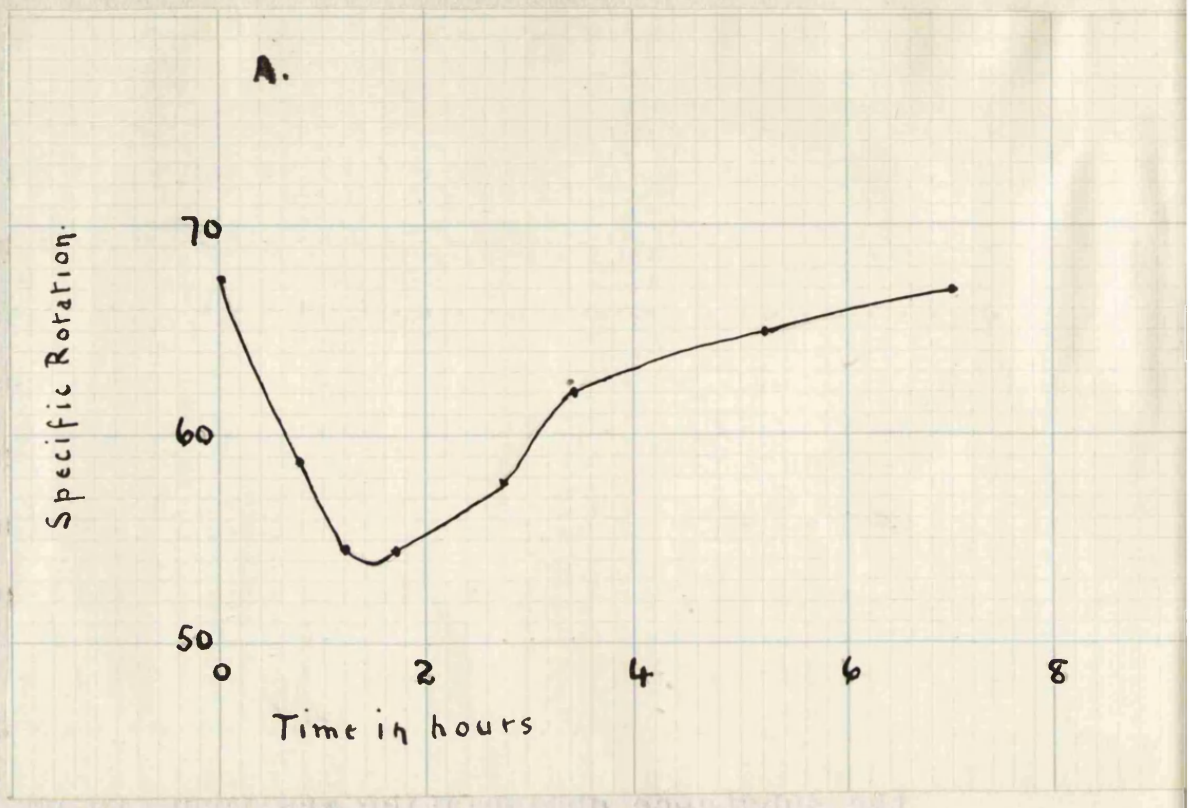
The above compound is isomeric with the trimethyl-fructose-monoacetone described by Irvine and Patterson, (J.C.S.1922,121,2146),and prepared by the methylation of fructose-monoacetone with silver oxide and methyl iodide.This compound,which was a mobile liquid, (B.P.135°-138°/10 mm.),had the following physical constants.

$[\alpha]_D$ in water = $-147.9°$ for c=0.902

$[\alpha]_D$ in ethyl alcohol = $-125.7°$ for c=0.887

$[\alpha]_D$ in acetone = $-125.0°$ for c=1.208

As the above reaction is a particularly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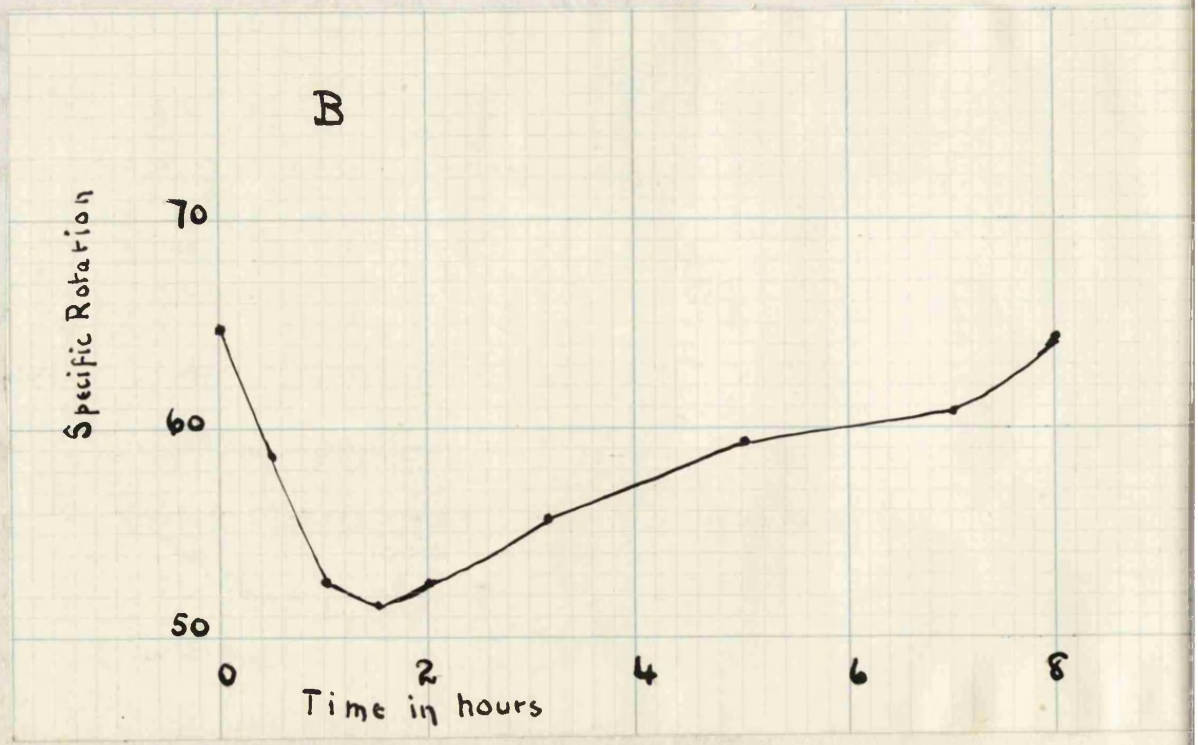
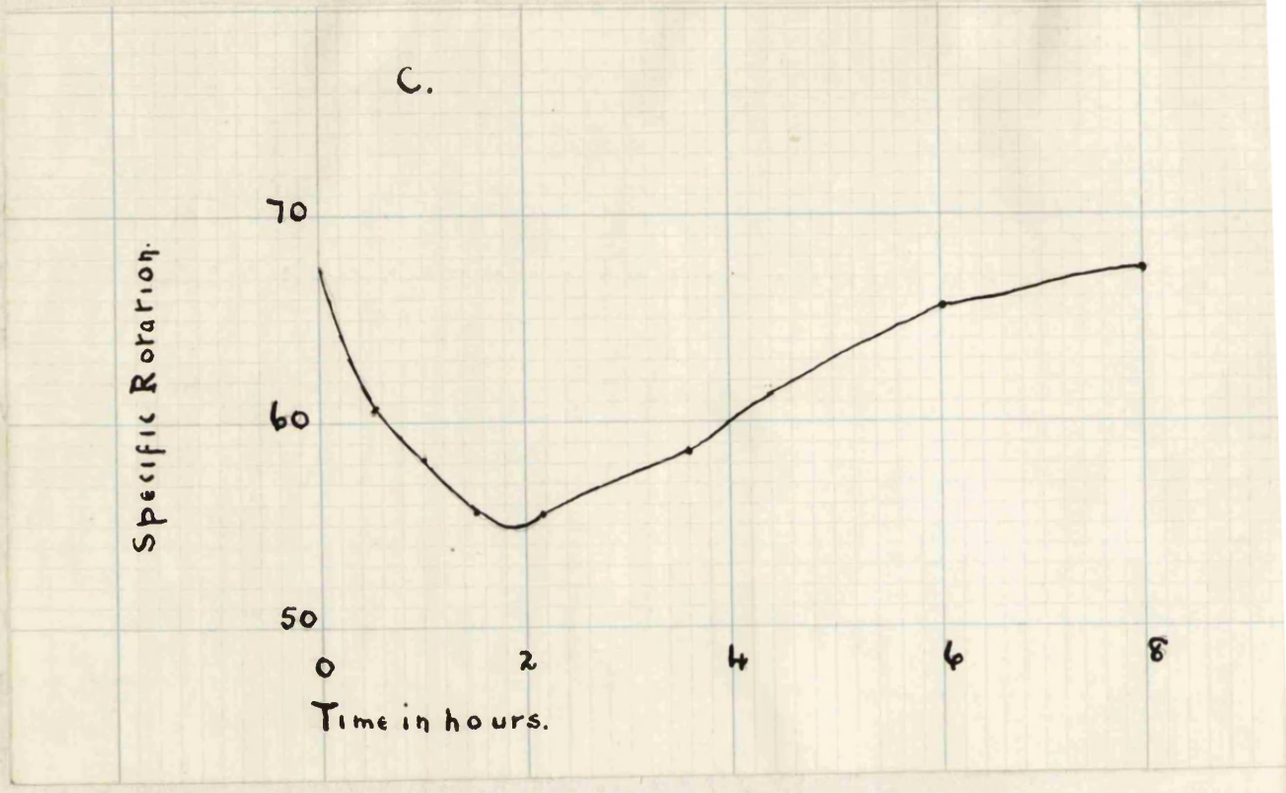

B
Specific Rotation
70
60
50
0
2
4
6
8
Time in hours

delicate one,in which differences in the rate of condensation would serve to indicate different trimethyl fructoses,it was decided to apply it to the various fractions obtained by distilling a quantity of trimethyl γ-methylfructoside.Such a distillation would serve to effect only an approximate separation of the different trimethyl γ-fructoses which might be present,as the boiling points of these would lie near one another. Differences between extreme fractions,however,would be detectable both in the optical rotations they displayed and in the rates of condensation with acetone.Four fractions were collected and the rotations of these were all in agreement.The rates of condensation with acetone are shown in the attached polarimetric curves,(A.B.C.D) where it will be seen that the different fractions are converted into the acetone derivative at the same rate and that this rate is in good agreement with that obtained when using trimethyl γ-methylfructoside which had not been fractionally distilled.

These results indicate that only one trimethyl fructose is obtained on hydrolysing trimethyl inulin.The yield of trimethyl γ-methylfructoside was approximately 90% of that required by theory.Considering the loss involved in the operations and particularly in such a process as distillation at the mercury pump, ~~this~~

C.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Specific Rotation.
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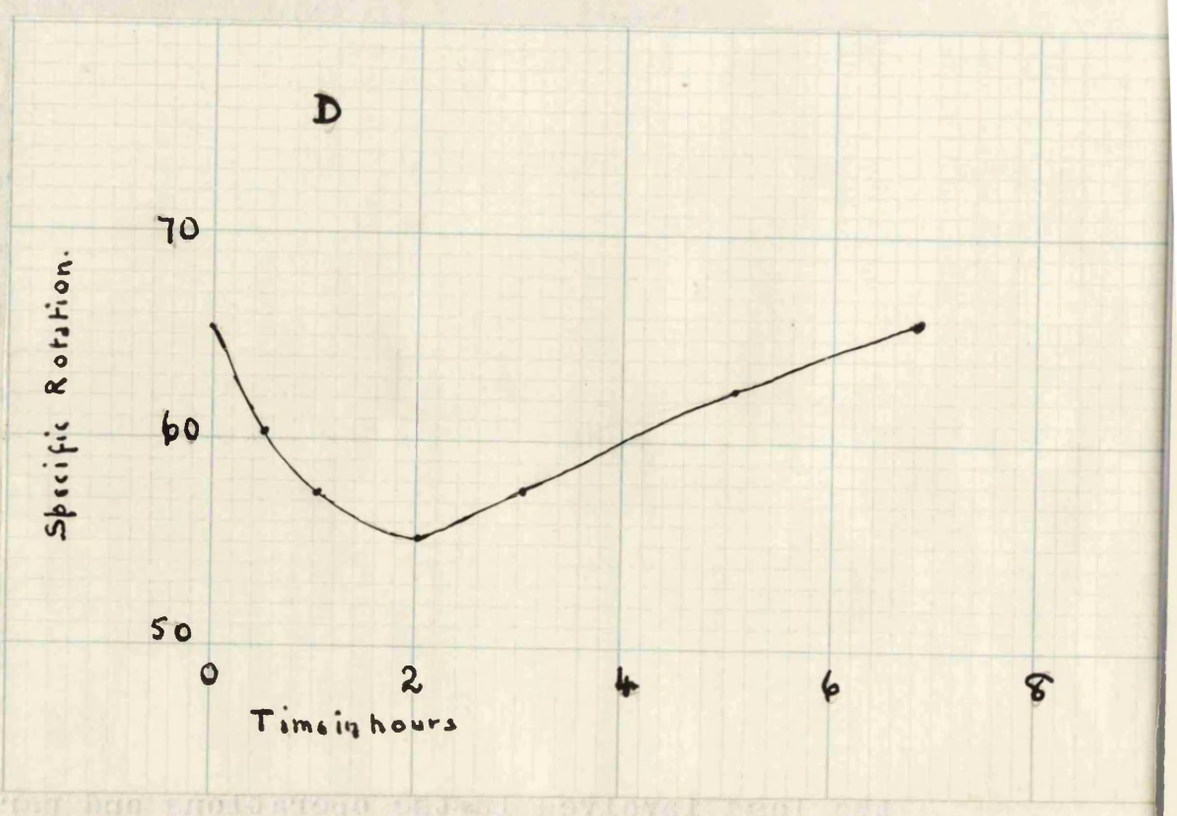
Time in hours.

D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Specific Rotation.
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8
Time in hours

this indicates that the reaction is quantitative and that the inulin molecule is symmetrical.

The oxidation of trimethyl γ-fructose and tetramethyl γ-fructose prepared from it was carried out in the manner described by Haworth.(J.C.S.1920,117,199) The treatment with nitric acid was prolonged at a temperature of 68° for 6 hours,which is the usual period allotted to similar reactions in the sugar group.In both cases,however,it was found on isolation that the products were not free from reducing compounds and,since the formation of aldehydic or ketonic acids cannot be regarded as probable in the presence of such an agent as nitric acid at the temperature employed,the conclusion was drawn that the oxidation of the sugar had not been completed under the conditions used,though the reaction in both cases had apparently ceased.

The oxidation of trimethyl γ-fructose was therefore repeated,a more prolonged treatment with nitric acid,(20 hours heating at 68°),being given.During the time this work was in progress Haworth,(J.C.S.1923, 123,294),has published results based on the application of the same method.After removal of the excess of nitric acid and on attempting to distil the product,it was found

impossible,despite repeated attempts,to maintain the high vacuum necessary to effect distillation.Though there was no obvious decomposition,this repeated change in the pressure could only have been due to the occurence of some side reactions and to this,with the consequent formation of degradation products,is to be traced the high values found for carbon on analysis by the combustion method of the final product.Despite the prolonged oxidation,the product still showed all the typical reactions of a γ-sugar and must have contained in addition to the ester of the acid some trimethyl fructose and trimethyl γ-ethylfructoside,the last being formed during the treatment given to effect esterification. No definite conclusions could therefore be drawn from the analysis of the liquid obtained on distillation.The boiling of this,135°-140°/0.35 mm.,is sufficiently near that of trimethyl γ-fructose,145°/0.4 mm.,to prevent a complete separation of these substances being made when dealing with comparatively small amounts of them.The essential product,however,was separated from the mixture of constituents in the form of the barium salt.Analysis of this showed it to possess the composition of a barium salt of a dimethoxy dihydroxy valeric acid.

An examination of the results of these

different oxidations indicates that the temperature employed, 68°, is not sufficiently high to effect complete oxidation of the sugar. Confirmation of this is found in the fact that, when the stable form of tetramethyl fructose was oxidised with nitric acid, (Irvine and Patterson, J.C.S. 1922, 121, 2696), a temperature of 86°-90° was found to be necessary, the reaction ceasing entirely at temperatures lower than 80°.

PART III.

EXPERIMENTAL PART.

The inulin used throughout this series of researches was prepared from dahlia tubers in the manner described by Irvine and Steele.(loc.cit.)

Preparation of Trimethyl Inulin.

The trimethyl inulin employed as starting material was prepared according to the method described by Irvine and Steele.In a typical experiment 32 gms.of finely powdered inulin were dissolved at 60° to 70° in 60 c.ccs.of 30% sodium hydroxide.After the solution had been cooled to 35°,it was maintained at this temperature and constantly stirred during the subsequent addition of 80 c.ccs.of dimethyl sulphate (3 mols.) and 200 c.ccs. of 35% sodium hydroxide (total 6 mols.)which were added slowly and simultaneously from separate tap funnels.At the end of three hours,when the addition was complete, the temperature of the water bath was gradually raised to 75° and finally to 100°,this temperature being maintained for thirty minutes to decompose any excess of methyl sulphate.

After cooling the solution,carbon dioxide was passed through it for a prolonged period,an equal

volume of rectified spirit added and carbon dioxide again passed.The precipitated inorganic salts were removed by filtration and washed with rectified spirit. The filtrate was neutralised with dilute sulphuric acid and,after filtering,the aqueous alcohol was removed by distillation under diminished pressure.The bulk of the product was obtained here but part remained behind with the inorganic salts which were extracted with boiling absolute alcohol.

With the product so obtained and without further purification,the same series of operations were repeated.The partially methylated inulin was freed from sodium methyl sulphate and inorganic residues by extracting with boiling chloroform.This solution was heated at the boiling point with animal charcoal for thirtyminutes, filtered and the chloroform removed by distillation.A pale brown glass,which could be powdered,remained;the properties of this are given in the scheme outlined below.

The further methylation of this material was continued by treatment with silver oxide and methyl iodide.30 gms.(the weight obtained from the original 32 gms.of inulin),were dissolved at the boiling point in 120 gms.of methyl iodide.75.6 gms.of dry silver oxide

were added gradually and the alkylation continued by boiling under a reflux condenser for eight hours.The product was isolated by extracting with hot alcohol, filtering and allowing the solution to cool.A white precipitate gradually settled out;this was separated by filtration,dissolved in chloroform and the solution boiled with animal charcoal for twenty minutes.After filtering and removing the chloroform by distillation, a colourless solid remained.This (A) proved to be trimethyl inulin.

Found. C = 52.84% H =7.95%

OMe = 46.00%

Trimethyl Inulin, $C_6H_7O_2(OCH_3)_3$,

Requires C = 52.94% H = 7.84%

OMe =45.8%

The alcohol,which had been used in extracting the silver residues was also distilled.The product was purified by extracting with chloroform,boiling this solution with decolourising charcoal,filtering and removing the chloroform by distillation.A pale brown syrup remained (B).

The above series of operations were twice repeated with separate quantities of inulin, the results of the methylations and properties of trimethyl inulin are tabulated below.

Results of Methylations.

	1st Exper.	2nd Exper.	3rd Exper.
Wt. of inulin used.	32 gms.	32 gms.	32 gms.
Wt. of product after two methylations with methyl sulphate and sodium hydroxide.	30 gms.	30 gms.	32 gms.
Methoxyl content after above methylations.	37.6%	40.02%	38.6%
WT.of trimethyl inulin.	27.5 gms.	21 gms.	24 gms.
Rotation in chloroform.	50.85 for c = 1.33	39.8 for c = 1.206	41.16 for c = 1.325
Softening point.	133-142	135	120-125
Solubility.	Soluble in chloroform and boiling ethyl alcohol. Sparing solubility in ether, ethyl alcohol in the cold and in acetone. Very slightly in water, insoluble in hot water.		
Wt.of product B.	0.5 gms.	5 gms.	3 gms.

As a general rule it may be stated that the trimethyl inulin with the higher laevo rotation was found to be more insoluble in alcohol and ether than that with a lower laevo rotation.

Examination of Product B.

From third experiment. Wt. = 3 gms.

This product,which effected no reduction of Fehling's solution until after hydrolysis,gave the following results on analysis;

C =50.9% H = 7.46% OMe = 39.16%

$[\alpha]_D$ = 29.19°

An attempt was made to increase the methoxyl content by methylating with silver oxide (7.5 gms.) and methyl iodide (12 gms.),the alkylation being continued as usual for eight hours.The product was extracted with chloroform,the solution decolourised by boiling with animal charcoal,filtered and the chloroform removed by distillation.A pale brown syrup was obtained OMe=40.66%

This was dissolved in 75 c.ccs.of absolute alcohol and 25 c.ccs. of water.To this 1.3 gms.of oxalic acid were added and the hydrolysis of the syrup completed by heating at the boiling point for ten hours.The solution was neutralised by calcium carbonate and,after filtering,

the aqueous alcohol distilled.On extracting the residue with ether,a quantity of resinous matter was left behind. The ethereal solution was taken to dryness and the pale yellow,mobile syrup remaining distilled at the mercury pump.It was collected as follows;

1st Fraction. B.P. 120° to 125° / 0.28 mm.

n_D = 1.463

2nd Fraction. B.P. 130° to 140° / 0.3 mm.

n_D = 1.470

An examination of the product (B) from methylation 2 gave similar results on hydrolysis and fractionation.

1st Fraction. B.P. 110° to 115°/0.25 mm.

n_D =1.4555

2nd Fraction. B.P. 124 to 127 /0.32 mm.

n_D = 1.4671

These distillates,from their boiling points, mobilities and refractive indices,were obviously tetramethyl fructose and a mixture of this with trimethyl fructose. (Tetramethyl fructose B.P. 110°-115° / 0.2mm. n_D=1.4545,Ph.D.Thesis,Dr.J.G.Mitchell;Trimethyl fructose B.P. 146° / 0.37 mm.,n_D = 1.4689,Irvine and Steele loc.cit.)

Depolymerisation of Trimethyl Inulin.

The following treatments were found to be without depolymerising effect on trimethyl inulin;

(a) boiling with acetone for four hours;

(b) heating at 120°to 130°/ 10 mm. for one hour;

(c) fusing and heating at 145°to 150°/10.mm.for one hour.A pale yellow melt was obtained,which solidified on cooling to a yellow glass.

(d) heating at 170°/ 0.15 mm.for three minutes.When the heating was continued for alonger period at this temperature or when the temperature was raised above this point,the material was completely decomposed.

In all of these cases the material was recovered unchanged in appearance,in solubility and in optical rotation.

(e) Oxalic Acid.

A 3% solution of trimethyl inulin in 99% ethyl alcohol containing 1% of crystallised oxalic acid was heated at the boiling point for twenty hours.The solution was neutralised by means of calcium carbonate, filtered and the alcohol removed.The residual syrup was extracted with ether and the solvent evaporated.Trimethyl γ-ethylfructoside and trimethyl γ-fructose,which had been formed,were removed by distillation at the Gaede pump,

the temperature of the bath being finally raised to 200°/ 0.35 mm.A small residue,consisting of a brown viscous syrup,remained which was insoluble in petroleum ether and effected practically no reduction of Fehling's solution until hydrolysed.

Rotation in chloroform, c = 0.731

$[\alpha]_D = +47.14°$

In a second experiment, c = 0.54

$[\alpha]_D = +38.18°$

An attempt was made to increase the methoxyl content by methylating with silver oxide and methyl iodide.This gave a negative result.

Found OMe = 45.72%

Trimethyl inulin $(C_6H_7O_2(OCH_3)_3$ requires OMe = 45.8%

(f) Trimethyl inulin was dissolved in chloroform and heated at the boiling point in the presence of charcoal, magnesium sulphate and a drop of sodium hydroxide respectively.The substance was recovered unchanged in each case.A similar result was also found when trimethyl inulin was dissolved in chloroform,the solution boiled and the chloroform removed by distillation,this treatment being repeated three times.

Found c = 1.3568 $[\alpha]_D = -47.10°$

Original rotation c = 1.8840 $[\alpha]_D = -50.85°$

(g) Several experiments were carried out in which inulin or trimethyl inulin was heated at a temperature of 160° in glycerol.In the former case the inulin was recovered unchanged on pouring the glycerol into water.In the latter case,when the glycerol had been removed by distillation at the mercury pump and washing with water,trimethylinu inulin was recovered with the rotation unchanged.

Trimethyl Fructose.

Five gms. of trimethyl inulin were heated at the boiling point with 100 c.ccs.of 99% ethyl alcohol containing 1% oxalic acid for thirty hours.The solution was neutralised by the addition of calcium carbonate, filtered and the alcohol removed by distillation.The residual syrup,consisting of a mixture of trimethyl ethylfructoside,trimethyl fructose and depolymerised trimethyl inulin,was dissolved in water containing 0.5% oxalic acid and heated in a water bath at 100° for four hours.The product was isolated as above,the syrup thus obtained being purified by extracting with ether, decolourising the solution by boiling with animal charcoal and,after filtering,distilling off the ether.3.5 gms.of a pale yellow,viscous syrup were obtained which had the

typical properties of a γ-sugar.It decolourised alkaline potassium permanganate,reduced ammoniacal silver nitrate giving a silver mirror and effected reduction of Fehling's solution,when heated or in the cold,with the formation of brick red cuprous oxide.After drying at 90° to 100° and distillation at the mercury pump,it gave the following analytic results.

Found:- C = 48.74% H = 7.97% OMe = 41.44%

Rotation in chloroform c = 0.879

$[\alpha]_D = +28.5°$

$n_D = 1.4686$

Trimethyl fructose, $C_6H_9O_3(OCH_3)_3$, requires

C = 48.75% H = 8.11% OMe = 41.88%

No crystalline osazone could be obtained when the substance was treated in the usual manner with phenyl hydrazine and acetic acid.The result was confirmed that a reddish brown syrup was formed,which may be a hydrazone.This was freed from phenyl hydrazine by washing the ethereal solution with acetic acid;on removing the ether,however,no crystalline product was obtained.

Trimethyl Methylfructoside.

2.1 gms. of trimethyl fructose were dissolved in 120 c.ccs. of dry methyl alcohol containing 0.25% of hydrogen chloride and kept at the temperature of the room. The course of the reaction was followed polarimetrically. The temperature was approximately 16°.

Time.	α for l = 1	$[\alpha]_D$
Zero	+ 0.41	+ 23.29°
5 minutes	+ 0.4	+ 23.25
30 minutes	+ 0.4	+ 23.25
60 minutes	+ 0.48	+ 27.31
120 minutes	+ 0.59	+ 33.75
3 hours	+ 0.66	+ 37.58
4¾ hours	+ 0.85	+ 48.37
7 hours	+ 0.93	+ 52.91
9¾ hours	+ 1.00	+ 56.90
24 hours	+ 1.07	+ 60.88
48 hours	+ 1.13	+ 64.30
60 hours	+ 1.13	+ 64.30

The reaction was thus complete at the end of 48 hours, when the solution no longer reduced Fehling's solution.

The solution was neutralised by shaking with silver carbonate and, after filtering, the methyl

alcohol was removed by distillation.The residue was extracted with ether and the solution decolourised by boiling with animal charcoal.After filtering and distilling the ether,1.9 gms.of a mobile syrup were obtained.This, on distillation at the mercury pump,yielded 1.56 gms. of a colourless,mobile syrup,B.P.108°/0.21 mm.,n_D= 1.4570. The boiling point and refractive index agree with the values given below for trimethyl γ-methylfructoside.The substance did not reduce Fehling's solution until after hydrolysis.It was therefore concluded that the substance was trimethtl methylfructoside.

Hydrolysis of Trimethyl Inulin and Simultaneous Formation of Trimethyl methylfructoside.

2 gms. of trimethyl inulin were added to 125 c.ccs.of dry methyl alcohol containing 0.5% hydrogen chloride and heated in a water bath at the boiling point.The material was insoluble in the cold and only went into solution gradually,so that it was impossible to take polarimetric readings at the commencement and in the early stages of the reaction.At the end of one and a half hours a constant rotation $[\alpha]_D$ = +66.25° was

obtained.The solution was then neutralised by means of silver carbonate and the product,isolated and purified as above,consisted of a colourless,mobile syrup, B.P.108°-110° / 0.3mm.,which reduced Fehling's solution only after hydrolysis.

Found; C = 50.74% H = 8.47% OMe = 51.65%

Trimethyl methylfructoside, $C_4H_8O_2(OCH_3)_4$

requires C = 50.85% H = 8.47% OMe = 52.50%

The substance was thus clearly trimethyl methylfructoside.

Rotation in methyl alcohol, c = 0.891

$[\alpha]_D = +65.05°$.

Condensation of Trimethyl Fructose with Acetone.

1.34 gms. of trimethyl methylfructoside were dissolved in 70 gms. of acetone containing 0.2% of hydrogen chloride.The solution was kept at the temperature of the room and repeatedly shaken,the following polarimetric readings being taken.

Time.	for l = 1	$[\alpha]_D$ T = 16 approx.
Zero	+ 1.02	+ 66.6°
10 minutes	+ 0.94	+ 61.38
30 minutes	+ 0.91	+ 59.42
1 hour	+ 0.87	+ 57.26
2 hours	+ 0.82	+ 52.55
4 hours	+ 0.93	+ 60.65
8 hours	+ 1.07	+ 68.38
9 hours	+ 1.07	+ 68.38

The solution was then neutralised by the addition of silver carbonate. After filtering off the silver residues, the methyl alcohol was removed by distillation and the syrup then remaining was distilled at the mercury pump. 1.3 gms. of a colourless syrup, B.P. 95° / 0.42 mm. were obtained.

Found, C = 54.74% H = 8.59% OMe = 34.09%

Trimethyl fructosemonoacetone $C_9H_{13}O_3(OCH_3)_3$

requires, C = 54.96% H = 8.39% OMe = 35.5%

The substance was thus trimethyl γ-fructose-monoacetone.

Rotation in acetone, c = 1.0820

$[\alpha]_D$ = + 69.77°

The trimethyl γ-fructose monoacetone obtained above, was dissolved in water containing 0.25% hydrogen

chloride.The solution became very cloudy as hydrolysis proceeded but this was apparently complete in 2 hours at 100°.

The product was isolated in the usual manner and was distilled at 140°-145° / 0.4mm.

n_D = 1.4684

Found, OMe = 42.57%

Trimethyl fructose, $C_6H_9O_3(OCH_3)_3$, requires OMe = 41.88%.

Rotation in chloroform, c = 1.3540

$[\alpha]_D$ = +26.54

Detailed Examination of Trimethyl Methylfructoside.

Proof that the compound is Homogeneous.

6.5 gms.of trimethyl inulin were hydrolysed by boiling with methyl alcohol containing 0.5% of hydrogen chloride as described above.The method of isolation and purification was similar to that previously described,except that in this case the material was collected in fractions on distillation at the mercury pump,each fraction being separately examined.

The material was collected as follows;

Fraction.A

Wt. = 1.3 gms.

B.P. = 120°/ 1.5 mm.

n_D = 1.4579

$[\alpha]_D$ = +66.72° for c = 0.623.

Fraction B.

Wt. = 1.4 gms.

B.P.= 105° to 107°/ 0.6 mm.

n_D = 1.4578

$[\alpha]_D$ = +68.25° for c = 1.114.

Fraction C.

Wt. = 1.83 gms.

B.P.= 104° to 107° / 0.56 mm.

n_D = 1.4581.

$[\alpha]_D$ =+67.48° for c = 1.230.

Fraction D.

Wt. = 1.5 gms.

B.P.= 107° to 110° / 0.64 mm.

n_D = 1.4584.

$[\alpha]_D$ = 44.74° for c = 0.95.

The fraction D was found to effect some reduction of Fehling's solution. It was therefore dissolved in methyl alcohol containing 0,25% of hydrogen chloride, the method of purification being similar to that described in the preparation of trimethyl methylfructoside from trimethyl γ-fructose. The rotation was then found to be $[\alpha]_D = +65.^\circ$ for c = 0.477.

All rotations quoted above are in ethyl alcohol. The optical values ascribed to fraction A were obtained after redistillation.

Condensation of Fractions A,B,C and D with Acetone.

These reactions were carried out in the manner described in the preparation of trimethyl γ-fructose monoacetone, the course of the reaction being followed polarimetrically.

Fraction A. 0.7806 gms. of trimethyl γ-methylfructoside were dissolved in 39 gms. of acetone containing 0.2% of hydrogen chloride. The temperature in all cases was 16 approx.

Time.	for l = 1	$[\alpha]_D$
Zero.	+ 1.07°	+ 67.14°
40 minutes	+ 0.94	+ 58.98
70 minutes	+ 0.87	+ 54.99
100 minutes	+ 0.87	+ 54.59
2 hours 40 minutes	+ 0.92	+ 57.73
3 hours 20 minutes	+ 1.00	+ 62.70
5 hours 10 minutes	+ 1.02	+ 64.00
7 hours 20 minutes	+ 1.04	+ 65.25

Fraction B. 0.9868 gms. of trimethyl γ-methylfructoside were dissolved in 49 gms. of acetone containing 0.2% of hydrogen chloride.

Time.	α for l = 1.	$[\alpha]_D$
Zero	+ 1.05°	+ 64.89°
30 minutes	+ 0.95	+ 58.72
60 minutes	+ 0.85	+ 52.53
90 minutes	+ 0.83	+ 51.30
2 hours	+ 0.85	+ 52.53
3 hours 15 minutes	+ 0.89	+ 55.00
5 hours	+ 0.96	+ 59.21
7 hours	+ 0.98	+ 60.56
8 hours	+ 1.04	+ 64.27

Fraction C. 1.49 gms. of trimethyl γ-methylfructoside were dissolved in 49 gms. of acetone containing 0.2% of hydrogen chloride.

Time.	α for l = 1.	$[\alpha]_D$
Zero	+ 1.09°	+ 67.66°
30 minutes	+ 0.99	+ 61.44
60 minutes	+ 0.94	+ 58.34
90 minutes	+ 0.88	+ 55.90
2 hours 10minutes	+ 0.88	+ 55.90
3 hours 40 minutes	+ 0.94	+ 58.34
6 hours	+ 1.02	+ 63.31
8 hours	+ 1.08	+ 67.04

Fraction D.

0.2876 gms. of trimethyl γ-methylfructoside were dissolved in 16 gms. of acetone containing 0.2% of hydrogen chloride.

Time.	α for l = 1.	$[\alpha]_D$
Zero	+ 0.94	+ 65.33°
30 minutes	+ 0.88	+ 61.16
60 minutes	+ 0.83	+ 57.55
2 hours	+ 0.80	+ 55.60
3 hours	+ 0.83	+ 57.55
5 hours 10 minutes	+ 0.90	+ 62.55
6 hours 40 minutes	+ 0.94	+ 65.33

Oxidation of Trimethyl γ-Fructose.

At first this was carried out inthe manner described by Haworth,(J.C.S.1920,117,199),with the exception that ,to avoid the amount of time necessary to effect complete removal of the nitric acid,the acid product was converted to the ester in the manner described below and isolated in this form.The product had the following properties.

B.P. = 135°/0.8 mm.

n_D = 1.4588

$[\alpha]_D$ in water = +37.18°, for c = 1.076

C = 48.53% H = 7.41% OMe = 49.2%

The product was neutral to litmus and behaved on titration with sodium hydroxide as a lactone ot ester.It also effected some reduction of Fehling's solution and was apparently a mixture.

The oxidation was repeated in the manner which Haworth has since described(Haworth and Linnell, J.C.S.1923,123,294).6 gms. of trimethyl γ-fructose were dissolved in 69 c.ccs. of nitric acid,specific gravity 1.2.The solution was heated at 86°for two or three minutes and then at 68°for 20 hours.The excess of nitric

acid was removed by maintaining the solution at 40°/15 mm. while water was continually introduced and distilled until 2500 c.ccs. had collected.The evaporation was then continued in a stream of alcohol,using at first rectified spirit and finally 99% ethyl alcohol until 2 litres had been collected.The liquid was then taken to dryness and maintained at a temperature of 40°-60°/0.3mm.for five or six days.On raising the temperature,however, with a view to distilling the liquid,it was found impossible to maintain the high vacuum.

The acid product was therefore esterified by dissolving in methyl alcohol containing 1% of hydrogen chloride and heating at the boiling point for 6 hours.The solution was neutralised by means of silver carbonate,filtered and decolourised by boiling with animal charcoal.After filtering,the liquid was taken to dryness,a clear mobile syrup being obtained.This was distilled as follows.

(a) a small,volatile first fraction,B.P.125°/0.4 mm.

(b) collected as the main fraction,B.P.135°-140°/ 0.35 mm.

(c) small fraction,B.P.145°-150°/ 0.75 mm.

On analysis the following results were obtained.

(a) C = 54.03% H = 7.51%

(b) C = 51.09% H = 7.75% OMe = 46.30%

C = 51.44% H = 7.85%

(c) n_D = 1.4561

$[\alpha]_D$ in water = +29.08°, for c = 1.582

(c) C = 51.11% H = 7.86% OMe = 43.17%

Further examination of the main product (b) showed that it consisted of a mixture of compounds. On titration with barium hydroxide it behaved as a lactone or ester, the absorption of alkali in the cold being very slow. It reduced alkaline potassium permanganate readily and Fehling's solution giving bright red copper oxide, restored the colour to Schiff's reagent and reduced ammoniacal silver nitrate giving a silver mirror.

The acid product was therefore separated from the other constituents in the form of the barium salt. 1.2 gms. were heated at the boiling point with excess of barium hydroxide(0.3N.) for 1½ hours. After cooling the solution, carbon dioxide was passed through it, the barium carbonate separated by filtration and the liquid taken to dryness. The solid residue was extracted with ether to remove any unchanged syrupy products and then with boiling absolute alcohol to dissolve the barium salt. The solution was filtered hot, filtration in the cold

proceeding very slowly.The ethyl alcohol was removed by distillation and the barium salt purified by again dissolving in alcohol and filtering to free it from traces of inorganic residues.On evaporating the alcohol to small bulk and adding ether,a colourless precipitate was formed. This was filtered and well washed with ether;on drying in a vacuum dessicator it formed a gum,which on further drying could be powdered.It was analysed as follows.

C = 32.01% H= 5.23% OMe = 21.27%& 20.43%

Ba.as sulphate = 27.62%

The barium salt of dimethoxy dihydroxy valeric acid, $(C_7H_{13}O_6)_2Ba$ requires,

C = 32.17% H = 4.97% OMe = 23.71%

Ba = 26.19%

A small amount of the lead salt was subsequently prepared by treatment with lead hydroxide similar to the above with barium hydroxide.It was found to have OMe = 20.45%.

$(C_7H_{13}O_6)_2Pb$ requires OMe = 20.91%.

Preparation and Oxidation of Tetramethyl Fructose.

3.2 gms. of trimethyl γ-fructose were converted to trimethyl γ-methylfructoside in the manner already described. After the requisite time and when the reducing action on Fehling's solution had disappeared, the product was isolated in the usual manner.

The syrup from the above condensation was then methylated by means of silver oxide and methyl iodide (8 gms. of silver oxide and 12 gms. of methyl iodide). The alkylation was completed by heating for 8 hours. The product was isolated by extracting with ether and purified by the customary methods. Tetramethyl γ-methylfructoside was thus obtained; this was then hydrolysed by making a 2% solution of the sugar in water containing 0.25% hydrochloric acid and heating this at 90° for four hours. The product was purified and distilled, (B.P. 118°-120°/1.6 mm.), 2.5 gms. being obtained.

Found, OMe = 53.32% n_D = 1.4536

Tetramethyl fructose, $C_6H_8O_2(OCH_3)_3$, requires OMe = 52.50%

The tetramethyl γ-fructose so prepared was oxidised by dissolving in 28 c.ccs. of nitric acid,

heating the solution at 86° for two or three minutes to start the reaction and then at 68° for 6 hours. No attempt was made to isolate the acid produced by the oxidation at this stage; it was dissolved in 100 c.ccs. of methyl alcohol containing 1% of hydrogen chloride and the solution boiled for 6 hours to promote esterification. After neutralising the solution by means of silver carbonate and separating the silver residues by filtration, the liquid was decolourised by boiling with charcoal and taken to dryness. A small quantity of a colourless, mobile syrup was obtained which distilled at 133°/0.9 mm. On analysis the following results were obtained.

C = 49.13% H = 7.69% OMe = 50.72%

It was found that the syrup was not homogeneous as it had a reducing effect on Fehling's solution. As the amount of substance was insufficient to admit of redistillation or for the preparation of salts of the acid, no definite conclusions could be drawn from the analytical results regarding the constitution of the compound.

PART IV.

Discussion of Results.

The foregoing results may now be critically examined to the elucidation of the three main problems with which we have been concerned.

(A) Trimethyl Inulin and Depolymerisation of Inulin.

The study of the results obtained by different investigators on the progressive methylation of inulin serves to show that the process is first arrested either at the dimethyl stage,(OMe=32%),or at some intermediate position between dimethyl and trimethyl inulin.(OMe=37%-39%)In the latter case the product consists of a mixture of these two substances which,on further treatment,yields a trimethyl product containing three methoxyl groups for each anhydro-fructose residue.

The isolation and identification of such a product as dimethyl inulin is important as,though the introduction of the methoxyl groups is generally hindered by steric effects,it serves to indicate that there is a distinction between two of the hydroxyl groups in inulin and the third.Since the product yields on hydrolysis a characteristic dimethyl γ-fructose,one of the positions masked by a methoxyl group must necessarily be that which,in the stable form of fructose,is engaged in the

formation of the butylene-oxide linking. It will be seen later that these facts are upheld by a study of the oxidation products of trimethyl γ-fructose.

As methylation progresses it may be assumed that depolymerisation increases. Karrer is of the opinion that this is not so but believes that the mere act of solution of the methylated product effects depolymerisation. Whilst it is difficult to offer any experimental evidence in support of either theory, the latter may be regarded as very improbable. There is a gradual change in solubility of the product, which depends in part on the molecular complexity and in part on the extent of the substitution. A similar change is found during the methylation of starch, where, with increasing methoxyl content, the characteristic colouration with iodine gradually disappears, indicating that the molecular complexity is being gradually decreased.

Regarding the composition of trimethyl inulin the crux of the problem lies rather in the question as to whether,

(a) the product that is obtained has a definite molecular complexity, between which and the final product of hydrolysis, no intermediate products can be detected,

(b) the depolymerisation is arrested at definite stages

corresponding with more or less stable molecular complexes,
or(c) there is a gradual transition from the more to the less complex aggregate.

The first point of view must be rejected, since it cannot be brought into agreement with the obtaining of a laevo-rotatory and dextro-rotatory trimethyl inulin.The marked difference in rotation,the behaviour with different solvents and the general appearance of these two compounds indicate that there is a difference in their molecular complexities.The latter compound could be distilled,though with some decomposition, showing that the molecular complexity was not very great and that the compound was of the nature of a trisaccharide. The former proved to be entirely non volatile but decomposed entirely on heating,so that the molecular weight was very much higher than that of the latter.

In the case of inulin itself similar phenomena occur.Pringsheim,in ' Die Polysaccharide' points out that,by the action of diastase,inulin is degraded to fructose but that intermediate dextrin like products have been observed,though none of these have so far been obtained in the crystalline state.

We are thus faced with the alternative

of (b) or (c). If (c) were correct, then we should expect and ought to be able to obtain trimethyl inulin with any rotation between two clearly marked extremes, i.e. -50° on the one hand and $+55^{\circ}$ on the other. The experimental evidence does not support this view, since no methylated products have yet been obtained with rotations, for example, of the order -20° or $+10^{\circ}$. This may be due to the instability of such compounds or the failure of the methods of separation adopted but it is much more probable that the compound, with the extreme laevo rotation, represents a definite stage of molecular complexity and that, with the extreme dextro rotation, another and less complex.

According to prevailing views on the structure of the polysaccharides, these consist fundamentally of simple ring structures, - an anhydro-trisaccharide in the case of starch and cellulose, - and the more complex product is obtained by the interlocking of the simple units. Inulin, as is known from molecular weight estimations of inulin triacetate and of trimethyl inulin, consists of nine anhydro-ketose residues. Evidence has already been given from the work of Pringsheim, (Ber. 1922, 55, 1414-1425), on the osazones obtained from inulin and on the additive compound formed, when inulin is treated with sodium ethoxide, that inulin is a product of a

polymerised anhydro-trifructose.Since it has also been shown that the dextro-rotatory product was of the nature of a methylated anhydro-trisaccharide and since the extreme laevo-rotatory product contains nine anhydro-ketose residues,it is not difficult to understand why there is no definite intermediate product between the two.

The laevo-rotatory variety of trimethyl inulin itself,however,shows differences in solubility, melting point and in rotation.It is true that the product is amorphous and not crystalline but such differences as have been indicated are too great to be explained on this basis.Whereas the variation in rotation is small in view of the nature of the compound, the other differences are quite marked.The compound with the lower laevo rotation was found to be more soluble in organic solvents and to have a lower melting point than that with a higher laevo rotation.These facts are, however,to be explained by the presence in the solid form of small and varying amounts of the more depolymerised form.This would account for the facts that it is possible to gather concerning such a substance.It would explain the decrease in laevo rotation with increasing solubility of the compound,since,as is well known and

as was seen in the course of the present research,the solubility of such compounds as trimethyl inulin varies in a marked degree when the solvent contains traces of such syrupy compounds as the dextro-rotatory form of tr trimethyl inulin.

If the view that has been taken of the methylation and depolymerisation of inulin is correct,- that the depolymerisation is arrested at certain stages corresponding to definite degrees of molecular complexity, then,if the methylated derivatives are more stable than the original polysaccharide from which they were obtained, it should be possible, by mild hydrolytic agency, to effect the degradation of the more to the less complex form.Despite much work and the expenditure of material which involved much time in its preparation,this expectation was only partially realised.The method used was to hydrolyse trimethyl inulin with a 1% solution of oxalic acid in ethyl alcohol and to stop the reaction before the hydrolysis was complete.The principal products of hydrolysis were methylated derivatives of fructose.The result,however,was quite definite that the first stage in the formation of these fructosides must be depolymerisation of trimethyl inulin as,when the process is arrested,a depolymerised form of trimethyl inulin is

obtained.The amount of this is small and in view of this fact,its rotation is in satisfactoryagreement with that of the dextro-rotatory form obtained on previous occasions.

The question that has been discussed is one of considerable difficulty,in view of the fact that inulin,being composed entirely of γ-fructose residues,is easily hydrolysed and that the intermediate products of hydrolysis are only isolated in small amount.

(B). Symmetry of the Inulin Molecule.

Condensation of Trimethyl Fructose with Acetone.

The proof of the symmetrical nature of the inulin molecule was adduced from an examination of the physical constants and rates of reaction of diff erent specimens of trimethyl γ-fructose.This examination, inaddition to showing that only one trimethyl γ-fructose is obtained on the hydrolysis of trimethyl inulin,also offers some indication of the structure of this compound and of the constitution of the products obtained from the condensation of fructose with acetone generally.

It is generally recognised that,in condensing with a polyhydroxy compound,acetone reacts in the ketonic and not in the enolic form.This condensation, as was shown by Irvine,Macdonald and Soutar,(J.C.S.1915-107,337),in an examination of glycerol acetone and the glycerol α-methyl ether derived from it,involves preferentially two neighbouring groups.Since trimethyl γ-fructose condenses with acetone,it would appear that one of the hydroxyl groups next to the fructosidic group must be free.

Mention has already been made of the fact that the trimethyl fructosemonoacetone,the preparation and properties of which have been described here,is isomeric with that obtained by Irvine and Patterson, (J.C.S.1922,121,2146).The latter compound was prepared from fructosemonoacetone and was shown,by hydrolysis of the isopropylidene residues and subsequent conversion of the laevo-rotatory trimethyl fructose so obtained into the stable,crystalline variety of tetramethyl fructose,(J.C.S.1918,113,257),to have no relationship to γ-fructose but to be derived from the stable form. In the series of transformations fructose - fructose diacetone - fructose monoacetone - trimethyl

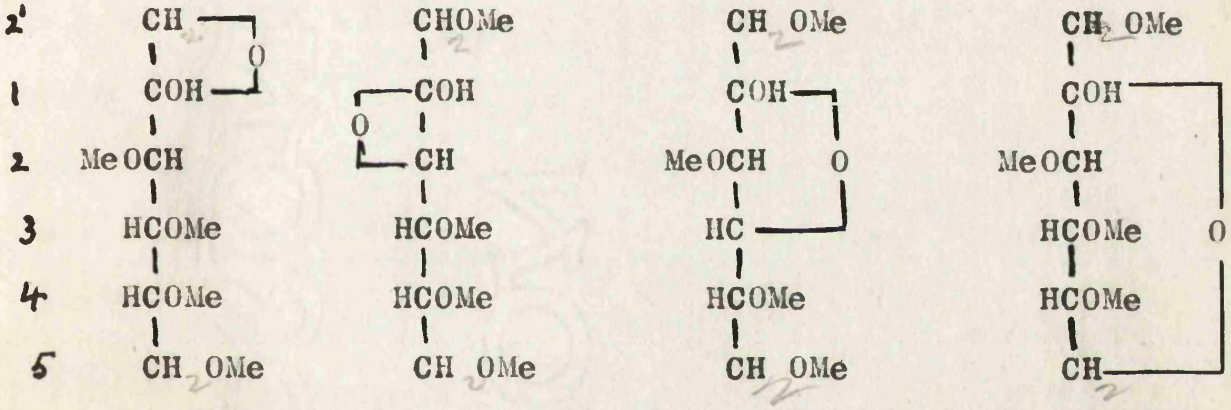
fructose-monoacetone - trimethyl fructose - tetramethyl methylfructoside - tetramethyl fructose,all the compounds are laevorotatory so that it is to be presumed that no rearrangement of the oxygen linking has taken place. The trimethyl fructosemonoacetone described in the present thesis,had a dextro rotation as had the trimethyl γ-fructose from which it was prepared and again gave on hydrolysis, so that no alteration in the position of the oxygen bridge is involved during the condensation with acetone. The properties of this compound thus offer valuable supplementary evidence regarding the constitution of the acetone derivatives of the stable form of fructose.The compound is of particular interest as γ-methylfructoside, when subjected to the action of acidified acetone,gave rise to the crystalline,laevo-rotatory form of fructose-diacetone,so that there would appear to be little tendency for the γ-sugar itself to condense with acetone.

(C) Oxidation of Trimethyl γ-Fructose.

It has already been indicated that the results of the oxidation of tetramethyl γ-fructose have led to conflicting views.The difficulty arises largely from the fact that only liquids and not crystalline

derivatives have yet been obtained as the essential products of oxidation.

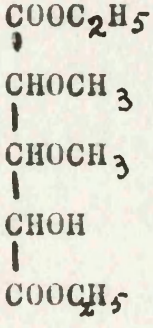
Tetramethyl γ- fructose must possess one of the four cyclic structures,



I II III IV

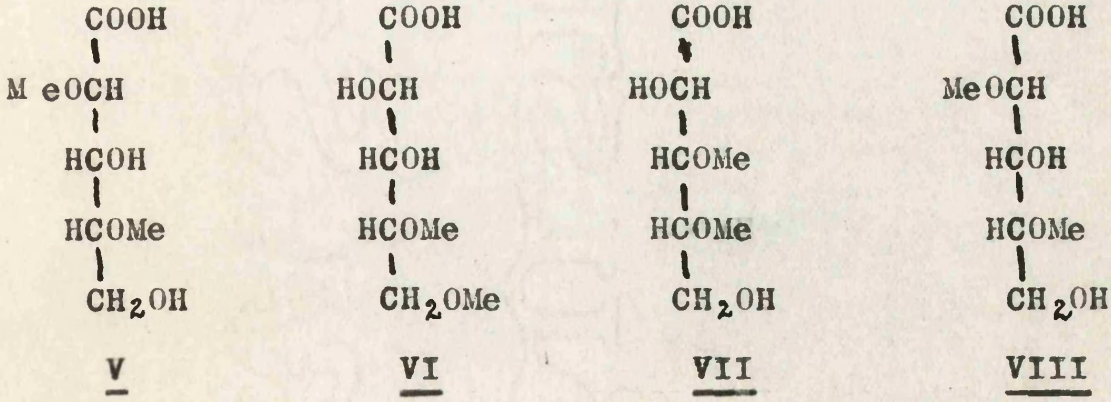
1-2'Ethylene Oxide. 1-2 Ethylene Oxide. 1-3 Propylene Oxide. 1-5 Amylene Oxide.

since,(Irvine and Patterson,J.C.S.1922,121,2696),it has been shown that the 1-4 or butylene-oxide structure is possessed by the normal,stable type.As,on the oxidation of this form,contrary to the usual experience of the scission of ketones on oxidation,the ketonic group remained with the larger residue,resulting in the formation of the diester of dimethoxy hydroxy glutaric acid,



it is reasonable to expect ~~that~~,using a similar oxidising agent,a similar acid with 5 carbon atoms in the chain in the case of the γ form.

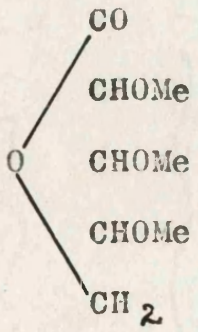
This expectation was upheld.The oxidation resulted,based on the analysis of the barium salt,in the production of a dimethoxy hydroxy valeric acid.From a consideration of the oxidation of a trimethyl fructose based on the structures III and IV,since it is highly improbable that γ-fructose possesses an ethylene-oxide linking,this acid must possess one of the structures.



Since dimethyl inulin on hydrolysis gives a dextro-rotatory dimethyl γ-fructose,it is known that the position 4 is occupied by a methoxyl group. Further the position 2' in trimethyl fructose must be masked by a methoxyl group,as the product of oxidation contains only two methoxyl groups.Since trimethyl γ-fructose condenses with acetone,a reaction involving two groups in spatial proximity,position 2 must contain

a free hydroxyl group.Of the above acids V and VIII are therefore to be excluded.

We are thus concerned with a choice of VI or VII.Haworth,from his study of the oxidation products of tetramethyl fructose,claims that the structure in γ-fructose involves an amylene-oxide linking and on this basis the acid obtained in this case should possess the formula represented by VII.The evidence produced, however,is not above criticism.It is difficult to understand how oxidation with such a drastic reagent as nitric acid could result in the formation of the lactone involving the potential primary alcohol group,

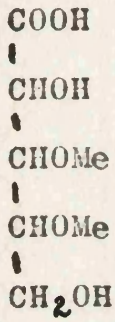


especially as this necessitates the assumption that the ring formation remains unruptured during the oxidation process.The question of the nature of the linking would have been finally settled had the lactone been wholly converted to the crystalline,corresponding acid which it is claimed,though supported by insufficient analytical data,settled out as a crystalline compound

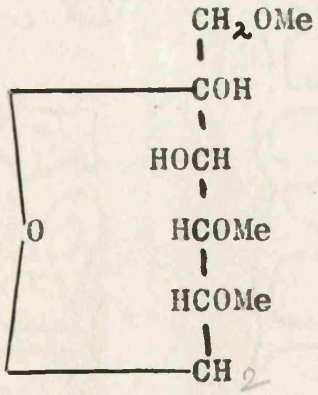
in small amount.The formula is,however,supported by the oxidation of the valero-lactone to glutaric acid which was recognised first in the form of the anhydride and whose constitution was confirmed by the conversion of the latter to the dimethyl ester.Here too it is to be regretted that the course of the reaction was not followed polarimetrically,so that the change in rotation on the opening of the anhydride ring to give an open chain compound could have been recorded.

The formula suggested by Haworth has this advantage over that of Boeseken,(Rec.trav.chim.1921,40, 354),suggesting a propylene-oxide linking.In the latter case,since the hydroxyl group 3 in fructose lies on the same side of the chain of carbon atoms as that in position 4,no change in rotation would probably be involved in the alteration of a 1-4 to 1-3 cyclic structure.On the other hand,in the case of the 1-5 oxygen linking,the groups attached to the carbon atom 5 being free to rotate,the oxygen bridge may lie on the opposite side of the chain of carbon atoms and thus influence the rotation.The amylene-oxide structure is further supported by the work of Helferich and Malkomes,(Ber.1922,55B,702),who have shown that δ-hydroxy-aldehyde exists as the cyclic or amylene-oxidic form.

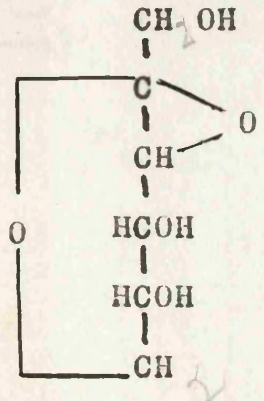
The weight of the evidence is thus in favour of the linking in γ-fructose being of the amylene-oxide nature, so that from the isolation of



the following structure is to be ascribed to the trimethyl γ-fructose derived from trimethyl inulin.



The anhydro-fructose concerned in the formation of inulin must thus possess the structure.



Such a structure is in agreement with all the experimental facts;

(a)the obtaining of a characteristic dimethyl inulin and from this of dimethyl γ-fructose;

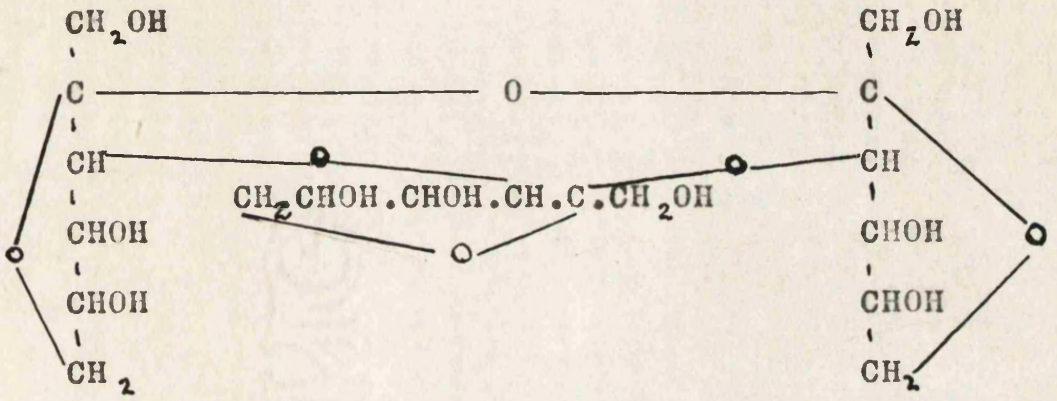
(b)the failure of trimethyl γ-fructose to give an osazone;

(c)the formation of trimethyl γ-fructosemonoacetone;

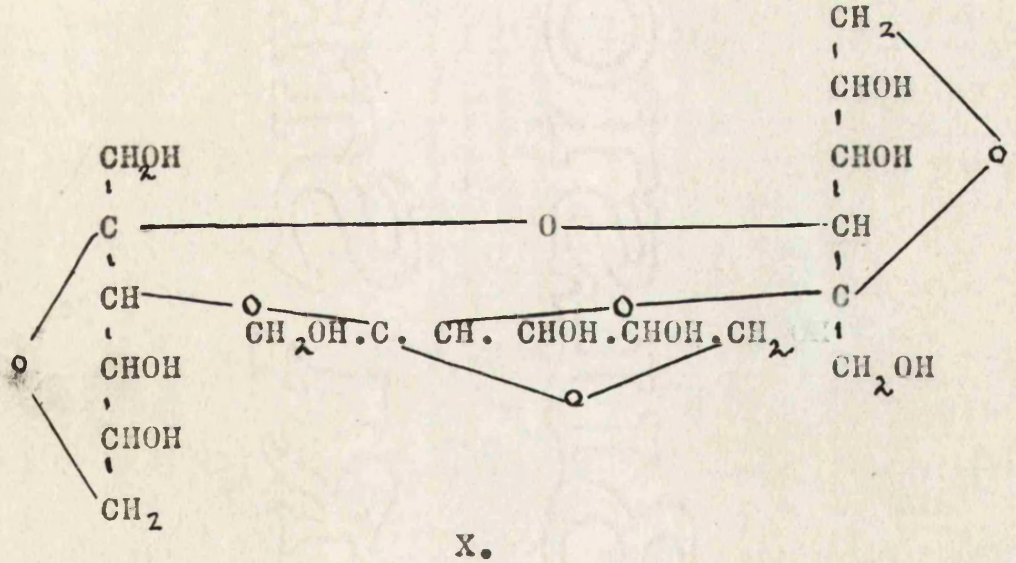
(d)the obtaining of a dimethoxy valeric acid from the oxidation of trimethyl γ-fructose.

Inulin must therefore be formed by the polymerisation of such a unit as this or,if the views that have been expressed regarding the depolymerisation of the compound are correct and Pringsheim's work on the additive compound formed when inulin is treated with sodium ethoxide accepted,this simple unit is involved in the formation of a higher aggregate such as an anhydro-trisaccharide and this in turn by polymerisation gives the complex polysaccharide.

Two different structures may be built up from the anhydro-fructose to represent the trisaccharide:-



IX.



X.

By the methylation process it is impossible to distinguish these possibilities. From the general nature of the linking found in the disaccharides and in the polysaccharides, so far as their constitution has been investigated, the structure represented by X, in which the reducing group is joined to a secondary

alcohol group,is to be regarded as the more probable.

In conclusion,the author wishes to express his gratitude to Principal Irvine,C.B.E.,F.R.S. under whose supervision the research was carried out.

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